Y61 x Y53

Segregation of Virus Resistance

Prepare inocula P21. Plate P22 — also surface

A24: - pick from colonies to minimal agar to avoid contamination
also test 15 directly.

		T1	T3	T5	T7	Lac B- M-	× T L B, Lac -
A	✓1	R	S	S	S		
	✓2	R ?	R	R	S	T_1 T_3 T_5 T_7	
	✓3	R	R	R	S		
	✓4	S	R	R	S	R S S S	2.
	5	?	R	R	S?	R R R S	4
	6	?	R	R	S	S R R S	3
	✓7	S	S	S	S	S S S S	2.
B	✓8	S	R	R	S	—	
	✓9	R	R	R	S	—	
	✓10	R	R	S??	S	R R S? S	1
	✓11	R	S	S	S	—	
	12	S?	R?	?	S	— ?	2
	13	R?	R?	?	S	—	
	✓14	S	S	S	S	—	

A1 and B1 were confused ? as demonstrated by Lac test!!
should be:

	S	S	S	S	—
	R	R	R	S	—
	R	R	R	S	—
A2.	R	R	R	S	+
	R?	R	R	S	+
	R?	R	R	S	—
	S	S	S	S.	—

and there is only one possible discrepancy! A5. marked — R.
otherwise: 10R/14.

	R	R	R	S	—
	R?	R	R	S	—
B	R	R	S??	S	—
	S	S	S	S	—
	?	R?	?	S	—
	?	R?	?	S	—
	S	S	S	S	—

Virus - Resistance segregation.

Y61 x Y53.

From same plating as 359:

	T1	T3	T5	T7	Lac
1	R	R	R	S	−
2	R	R	R	S	−
3	S	S	S	S	−
4	R	R	R	S	−
5	S	S	S	S	−
6	R	R	R	S	−
7	S	S	S	S	−
8	R	R	R	S	+
9	S	S	S	S	−
10	R	R	R	S	−
11	R	R	R.	S	−

	T1	T3	T5	T7	Lac
1	R	R	R	S	−
2	R	R	R	S	−
3	R	R	R	S	+
4	R	R	R	S	−
5	S	S	S	S	−
6	R	R	R	S	−
7	R	R	R	S	+
8	R	R	R	S	+
9	S	S	S	S	−
10	R	R	R	S	+

D

1	S	S	S	S	−
2	R	R	R	S	+
3	R	R	R	S	−
4	S	S	S	S	−
5	R	R	R	S	−
6	R	R	R	S	+
7	R	R	R	S	−
8	S	S	S	S	−
9	R	R	R	S	+

X

1	S	S	S	R S	−
2	R	R	R	S	−
3	S	S	S	S	−
4	S	S	S	S	−
5	S	S	S	S	−
6	S	S	S	S	−
7	S	S	S	S	−
8	S	S	S	S	−
9	S.	S	S	S	−
	R	R	R	S	−

A

1	R	R	R	R ↓ ?	−
2	R	R	R	R	−
3	R	R	R	R	+
4	S	R?	R	R	−
5	R	R	R	R	−
6	R	R	R	R	−
7	R	R	R	R	−
8	R	R	R	R	+
9	R	R	R	R	+
10	R	R.	R	R	−

C

23 R | 40. all T_1^R are $T_3^R T_5^R$

T_1^R Lac −	T_1^R Lac +	T_1^S Lac −	T_1^S Lac +
21	11	18	0

Virus resistants.

Streak out to purify:

Y63 Y53/Muc from A.

Y64 Y53/1 from A.

Y65 Y10/1/7M from C.

Y66 Y53/3,1,5,7M from D.

Y53/3,1,5,7S from D.

Y67 ~~Y53~~/7 M from E

Y68 Y53/7 S from E

After Y-platings, tested again 12/10.

		T_1	T_3	T_5	T_7	Morph.	#
A	Y57	R	R	R	S	Y53/3	M
	Y58	S	S	S	S	Y53/7	SR
	Y59	R	R	R	S	Y10/1/7	SR
	Y61	R	R	R	S	Y40/7	SR
	Y62	S	S	S	S	58-161/3	SR
	58	S	S	S	S	✓	SR
D	Y63	S	S	S	S	"Y53/1"	SR
	Y64	R	R	R	S	Y53/1	SR
	Y65	R	R	R	R	Y10/1/7	SR
	Y66	R	R	R	S	Y53/3	SR
	Y67	S	S	S	S	Y53/7	M
	Y68	S	S	S	S	Y53/7 S	M

also ~~#~~ T~~4~~, T6 R. probably contaminant.

Prepare plates for:

58-161/3

58-161/7

Compare $\frac{B\bar{B}}{B\dagger} = \frac{5D}{212}$ with $5/20$ on p. 364.

but need better information.

5 hour cultures, washed, mixed, and plated into various media.

Turbidity	Medium.	Colonyets.	Mean	m.d.	Excess.	R/prot.
±	O	217	212 ±34			1.00
	O	193				
	O	279				
	O	234				
	O	137				
++	B_1	760			548	2.58
	B_1					
−	B	100		d.u.u. note: thin agar layer	50?	<< 1.
		282				
±	L	421	389		177	.85
		367				
+	L	304	350		148	.65
		395				
++	M.	0 ! !		Does not seem to be so turbid that serum should be inhibiting ! !		
				Repeat & added proteoliptes.		
+	BB_1	764.			0.	
	BTL				—	
	ML	0				
	MT	0				
++	BLB_1	v. small cols.			?	
	BTB_1	+++			—	
	MB_1	0				
	MTL	0				
	MTB_1					
	BL				—	
	BT.				—	

Reversion controls

			stab.
Y53 ui:	TL.	0	++
	TB$_1$	12	+
	LB$_1$	2	+
Y40	M	0	++
	B	0	+

Conclusions:

Plate count determinations may be in error due to variable increases in cell density. B$_1$ seems to be a limiting factor in synteplasm. (Try it in aB$^+$ x bB$^+$.).

B$_1$ independent, or linked to: B$^+$; M$^+$

B linked to M.

L independent?

T. independent or linked to L.

∴ B$^-$ should be linked to Lac

and in this cross, we may find that the B$^-$ acted Lac + compared to B$^+$.

Similarly ē B$_1^-$

5/20 B⁻ Exp. 10.

5	15
10	10

$\chi^2 = \frac{25}{10} + \frac{25}{10} = 5$

$p = .025$. Need more data!

Recotypes Y40 x Y53. 364

Test colonies on ●, B, B_1; T; L medium appropriately & segregate together various single mutants for lysis & ferm. tests.

~~1?/31 L− ??~~

		T_1^R Lac−	T_1^R Lac+	T_1^S Lac−	T_1^S Lac+
		17	9	13	1
46/48	B_1−	~~26~~	~~2~~	~~20~~	~~0~~
2/30	L−	2	0	0	0
~~5/20~~	~~●~~ B−	2	2	0	1
~~4/76~~	~~●~~ T−	2	2	0	0

17	9	13	1
100	55	50	4.

all recotypes of B_1, Lac, T_1^R.

~~Prototrophes: See 362.~~

Prototrophes.

Ser 1.	4	4	6	0
Ser 2.	~~25~~ 24	17	15	0
	28	21	20	0.
Summary. Y. 357:	51	23	11	4.
B_1− may be deficient in T_1^R Lac+ class (parental type). Y. 359	79	44	31	4
	21	11	18	0
	~~95~~ 100	55	~~45~~ 50	4

Prepare medium P 2 4.

Plate Y 55 (lactose – from Y 53 + Y 40) into lactose – minimal.

10^4 colonies tooth

Galactose mutant

366

Irradiate 58-161 P24. 2 mins. Incr 2 ml 110 YB. Quad 1 min
Benzer.

20-24,000 colonies examined.

No typical gal- colonies. Several sectorial colonies + some rather mucoid gal- were seen.

Streak out on ~~lactose~~ EMB.
galactose

No mutants.

1 mucoid form.

Penicillin-resistance.

Y53 x Y54. grow separately. Pick prototrophs

1. ferments.
2-16 non-ferments.

give to Truffaut.

Y10/1 x453 x 58-161.
"3"

November 24, 1946.

(Naguoutb.) test prototypes.

38 T_1^S Lac$^-$
16 T_1^R Lac+

no { T_1^R Lac+
T_1^S Lac+.

Dry conditions. Y4C x Y53. 369.

26 NOV 1940

a) Synthetic medium preparation (T+B, B4); —

much more turbid; no protoplasts. Occ. on surface. ca 10^8:...

b) YB. 10^{-7} on surface. as many in deep agar.

suggests YB better than synth. However, must be repeated!

stat

11/24/46.

Y40 x Y53. Plate is growth into B_1.

Pulete H_2O; test on B_1+, B_1-.

33/39 = B_1-

6/39 = B_1+ = ~~[illegible]~~. 15%.

Test B_1^- for Lac, T_1.

12/3/46: Tests:

T_1^R Lac -	T_1^R Lac +	T_1^S Lac -	T_1^S Lac +
1	3	3	—
6	3	1	—
2	1	2	
4	1	2	—
13	8	8	0

T_1^R	T_1^S	Lac +	Lac −
		6	4

3-way crosses, etc.!

26 NOV 1946

a) BTL.

BT }	0.	0
BL }	B	10^2 turbid.
BT }	0	1 !!
TL }	T	≠ 0
BL }	0	0
TL }	L	10 ? T
BT+TL+BL	0	0 1 plaque ??
	0	0. Three plaques!??
	B	10^2 turbid
	T	0
	L	0 ?

Plaques are probably abortive and turbid.

b) BTB.

BB_1 }	0	
BL }	B	? turbid.
BB_1 }	0	0
B_1L }	B_1	0
B_1L }	0	0 — clear not turbid
BL }	L	1?
3	0	0.
~~BT+TL+BL~~	0	
	B	10^2 1 plaque?
	B_1	0
	L	plaques??

$BB_1 \times TL$. 0.

December 4, 1946.

YS3 × Y0 in YB. (5 growth) plate in vaccois + test. Compare ē
do. grown in Nutrint Saline Glucose.

NB } 0
NB } 0. ✓ 10^2 just as good as YB.

YB:
0
B
B
B
B_1
B_1
B_1
$\bar{T}$
T
$\bar{T}$
T
L
L
L
L
BLT
BLT
BLT
BLT. } most colonies do not resemble E. coli. Biotin is took seems contam.

$\bar{B}_1$	$\overset{+}{B}$	$\overset{+}{M}$	Lac−	T_1^S	$\bar{T}$	$\bar{L}$
B_1	B	M	Lac+	T_1^R	T	L
+	−	−			+	+

↑

Look for types which are $B_1^+ M^+$, i.e. crossover occurs and study progeny.

December 5, 1946.

$10^3/10^9 = 10^{-6}$!

YB. a) Plate Y40; Y53 on B_1 plate. Select colonies and plate entire multiplied colony into BMTL. If any colonies appear they may be either BM or the complementary recombinant.

P5. Test for linearity any ...L- should be tested thoroughly. [Use detection procedure?]

$$\frac{\vec{B}_1 \;\; \overset{+}{\vec{B}} \;\; \overset{+}{\vec{M}} \;\; Lac- \;\; T_1^S \;\; \bar{T} \;\; \bar{L}}{B_1^+ \;\; B^- \;\; M^- \;\; Lac+ \;\; T_1^R \;\; T^+ \;\; L^+}$$

complementary type is $\bar{B}\,\bar{M}\,\bar{T}\,\bar{L}$ and may have any lac, T_1 configuration, particularly Lac+ T_1^S.

b). Assuming that M is relatively far from L or T, so that (as in 4-strand) 2 double exchanges can be expected to occur in this region, plate out for such an exchange (e.g. −M, −T or −M −L [$\underline{BB_1L}$; $\underline{BB_1T}$] and examine for heterogeneity in lac. or T_1 (particularly the former).

BB_1L: as ~~above~~ + v. turbid. do not use!
below (371)

Linkage –

Compare B(P) x Y53 in (P) B_1 (cancel P- with proline)

and BB, x TL.

12/5/46. BB, x TL.

Repeat. 12/9/46.

A. BB, x TL.

0 ~~4to~~ 3 colonies. ?? coli.

B_1 No prototrophs. ~~4t~~

B. B x TL B_1.

0 } No colonies.

B_1 } rather turbid!

~~Inbreeding~~. 4 strand test

December 9, 1946.

Y40 x Y53: into BB, L. (A) and BB, T (B)

e.g. like 375

12/9/46.

Cultures ca. 8 hours.
(too old???)

Y40xY53. into BTL.

ca. 10 colonies. Latter inhibited by tumbril growth.

6315 x Y53.

December 9, 1946.

Y53 x 58-6315. (Biotin – "δ-alanine?" + cystine, 48hr.)

Gave a very high frequency ($5 \times 10^3 / 10^9$) of prototrophs; ca. same number of colonies on a δ-alanine plate. To Carl

~~Test prototrophs on T₁-Cal plates.~~ Carl – found δ-...+ types indicating separability of δ, cyst. ag.

Y4③ x Y53

$T^- + L^-$

10/5 - 10/46.

Plate on T, L resp. test do. Need more data.....

T: 5/20. $T_1^R Lac-$ $T_1^R Lac+$ $T_1^S Lac-$ $T_1^S Lac+$

(.25) 3 0 1 1

2 2

5 2 1 1 T^-

Of 364 4/26.

9/46 T- ✓ 1 ✓ ? 2 ✓ ++ ng 3 ✓ ++ n.f. 4 ✓ 5 ✓ ✓ ✓

T^+ also all ++.

∴ T^- $T_1^R Lac-$ & T^- $T_1^S Lac+$

L: 3/26. 1 2

(.11)

Of. 2/30. Both $T_1^R Lac-$ 2 0

364.

3 2 0 0

5/56.

n.g. (?) ✓ 1 ✓ 2 ✓ 3 ✓

$T_1^R Lac+$ (2).

~~LT talk~~

ca 10% L^- OK.

ca 10% T^-

10 December 1946.

8 Dec. plate colonies of (Y53 x Y40) from B_1 agar, into BTLM agar.

Most plates have 1-200 colonies, & many non-proliferating B_1^- in between.

12/10/46. Pick colonies a) to BLTM; BTM small tubes 10 tubes x 10 plates.

b) to BLTH large tubes (for detection plates)

Tests. (only BLTM+ BTM- or ? recorded).

Plate no:		T_1^R Lac-	T_1^R Lac+	T_1^S Lac-	T_1^S Lac+
	1		0		
	2		0		
	3		0		
	4		0		
	5		0		
	6		1		378-1
	7		1		-2
	8		1		-3
	9		2		-4, 5
	10.		2		-6, 7.

c) Pick colonies to EMB lactose (1 plate):

15 + (8)
4 - (9)

1 BM
2 BM
3 BM
4 BM
5 BM
6 B M+?
7

December 13, 1946. Plate following asiridin.

A 1. Y53 x Y40. (Shows)

B 2. Y64 x 58-161

C 3. Y65 x 58-161. (Y10 4/7 x 58-161).

D ~~GT.~~

4. Y67 x Y40

E 8. Y53 x ~~58 161~~. Y68.

F 9. Y67 x Y68.

most mocida too large

Best method: surface spreading!

A: Yield rather low! B. too turbid. BTL OK but >> than O.

B: also too heavy. V. low yield.

C: (O. none B_1: ca 20 = very wide zones of stimulation) all dup. (contam?)

D OK when sus. many more.

E ca 10^2-10^3 colonies. Not very much like coli, but test on EMB lac all mucoid, inc +

F O.

December 16, 1946.

12/16. Use B_1^- / BMTL plates of Exp. 378. Pick colonies from <u>lettered</u> plates to EMB lac to eliminate lac+ which from 378 are probably B−M−.

Streak out <u>lac−</u> colonies on EMB-lac to obtain pure cultures & avoid pitfall of dysgenesis. Test on:

~~BMTL B_1MTL B_1BTL B_1MTL B_1BMT~~ | ~~BB~~ M_2

~~BMTLB_1 BMTL BMB$_1$ TLB$_1$~~

		−B	−M	−T	−L	−B_1
	C1	+	+	+	+	−
	C2	+	+	+	+	−
lac−	D1	+	−	±	+	+
	E1	+	+	+	+	−
	E2	+	+	± − +	+	+
	F1	+	+	+	+	−
	F2	+	−	+	+	+
lac+	G1	−	−	+	+	+
	H1	+	+	+	+	−
lac+	J1	−	−	+	+	+
	K1	+	+	+	+	−
	M1	+	+	+	+	−
	378-8	−	−	+	+	+
	378-9.	±	+	+	+	+

+B_k

A. 14/15 +

B. none grew

C. 8/8 − < 1, 2

D. 1/1 − −1 ? B−M− lac−?

E 17/17 − < 1, 2

F 8/8 − < 1, 2

G ~~11~~ − 1 (lac+)

H 4/14 1

J 1/1 − 1

K 6/6 − 1

L ———

M 1/5 − 1

15.

Retest: 380 − D1
380 − E2 (on 0)
380 − F2

no variability is <u>streaked plates.</u> rec. in colony type.

378-8
378-9.

See 380.

	—	Lac	Small colonies.	✓
C1	B1	−	−	E1
C2	B1	−	− ?	
* D1	M (T)	− ✓	−	H1
E1	B1	−	✓	K1
* E2	(T)	−	− ?	
F1	B1	−	− ?	M1
* F2	(B) M	−	− ?	
G1	BM	+	−	-!
H1	B1	−	✓✓	C2
J1	BM	+	—	
K1	B1	−	✓	E2
M1	B1	−	✓	F1
378-8	BM	+		F2
378-9.	(B?)	−		

Pick <u>small</u> colonies to coli∞, [illegible]

<u>4 h at</u>

checks on	O	B	M	T	BM	TM
D1	+		+	+		+
E2 pr	+			+		
F2.	+	+	+		+	

	Type		−B1	−M	−T	−L	−B.
1	C2		−	+	+	+	+
2	K1		± +	+	+	+	+
3	E1		−	+	+	+	+
4	E1		−	+	+	+	+
5	E1		−	+	+	+	+
6	F1		+	+	+	+	+
7	E2		+	+	+	+	+
8	F2		+	±	+	+	+
9	F2		−	−	±	+	−
10	F1		−	+	+	+	+
11	H1	B1	−	+	+	+	+
12	H1	B1	−	+	+	+	+
13	K1	B1	−	+	+	+	+
14	[illegible]	B1	−	+	+	+	+
15	K1	B1	−	+	+	+	+
16	K1	B1	−	+	+	+	+
17	M1	B1	−	+	+	+	+
18	M1	B1	−	+	+	+	+
19	M1	B1	−	+	+	+	+

?? hold! → ++++ / ++++

8 F2	−	−	−	−	+	+	+	+	−	(+)
9. F2.	−	+	−	±	−	+	+	+	−	±

Dye - inhibition of E. coli. 38 c

January 5, 1947

1 ml, 36 hour broth cultures in YB agar

+++ = uniform turbidity.

		A	B	C	D
Proflavine	$1:10^3$	+++	———	———	———
	4		"		
	5			"	
	6				"
	7				

n.g. at all from both

Crystal Viol.	$1:10^3$	———	do.	do.	ca 10^3 cols.
	4	+++	———	———	———
	5		"		
	6			"	"
	7				

Y40 1 ml A
Y40 .1 ml B
Y53 1 C
Y53 .1 D

Proflavine is n.g. under these conditions
Survival ± crystal violet is OK in range 10^{-4} to 10^{-3}.
This should be extended. Uninoculated cultures?

January 9, 1947.

Irradiate in flask, varying times. Grow YB-1 ml Also plate on EMB .01 ml irradiated cultures

	S /100	S.	pS.	
0	+++			
15 sec	+++			
30	++±			
60	ca n×10³	10⁵		3?
120	ca p×10³	10⁵		4
300	0?/5	10³		6

1 min

pS/10 - ca 1/15 sec.

i.e. each 15 sec. kills 90% of the previous survivors.

non-[illegible] kill?

P10 Dilute 120 sec. 1:10⁷ on EMB plates + spread.

P14. Pick colonies which seem to be non-papillate. Sample is not clear-cut because plates are crowded and entire population could not be screened. Estimate ca 5-10×10⁴ tested 50,000

to fresh EMB for further test.

Pick 6 colonies to YB slants which seem to be non-papillate. 1 is mucoid.

See 416